Cytogenetic Analysis of Mehr Cotton Cultivar and Its Crossing Progenies: A Search for Unreduced Pollen Grains

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Received August 26, 2011; accepted January 12, 2012

Summary Cotton is cultivated in Iran with diploid and tetraploid forms and hybridization is a means to increase the genetic diversity and obtain new elite cultivars in this crop. The present study considers cytogenetic study of the Mehr (*Gossypium hirsutum*) cotton cultivar and its crossing progenies. The cultivars showed bivalents, univalents and quadrivalents in metaphase of meiosis I. They differed significantly in mean values of chiasmata formation and chromosome pairing due to genetic difference. Different types of meiotic abnormalities like chromosome stickiness, laggard formation, micronucleus, tripolar and multipolar cell formation, cytomixis and unreduced pollen grain formation occurred in the parental genotypes and their hybrids. Grouping of the cultivars based on cytogenetic features showed the effect of parental genotypes on the meiotic behavior of their crossing progenies.

Key words Hybrid cotton genotypes, Meiosis, Unreduced pollen grain.

Cotton with its diploid (*Gossypium herbaceum*) and tetraploid (*G. hirsutum*) cultivars constitutes one of the most important crop plants of Iran cultivated in many regions of the country. Tetraploid cotton (*Gossypium hirsutum*) has a genome constitution of AADD ($2n=52$) (Menzel and Brown 1978) and is one of the worlds most dominant cotton cultivars.

Hybridization is the main breeding strategy in cotton plants with the aim to broaden the genetic diversity available and produce new genotypes. Due to continuous artificial selection and cultivation of cotton cultivars, genetic erosion happens and useful genetic loci may be omitted from the germplasm. This could result in vulnerability to pests and diseases (Sheidai *et al.* 2008, Van Esbroeck and Bowman 1998). Therefore, making new hybrid combinations and studying the amount of genetic diversity obtained is important.

Our previous cytogenetic studies on cotton involved other cotton cultivars and they were mainly concerned with chiasma frequency and distribution (see for example, Sheidai *et al.* 2008, Tafvizi *et al.* 2010, Sheidai and Dezfolian 2008, Sheidai *et al.* 2006). However the present report considers cytogenetic characteristics of the Mehr cultivar and its crossing progenies for the first time with special attention to unreduced ($2n$) gamete formation.

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Materials and methods

Plant materials

Twelve cotton genotypes (*Gossypium hirsutum*) including the parental genotypes Mehr, Bellizovar, No. 200, Siokra, Sindose, Sahel and Tabladilla and their crossing progenies obtained by crossing with Mehr were cultivated. The cotton genotypes were cultivated in 3 rows of 10 m length with 20 cm interplant distance, in the experimental field of Gorgan Cotton Research Center of Iran, according to a completely randomized design (CRD) with 3 replications.

Cytogenetic analysis

For cytogenetical studies, 50 flower buds were used randomly from 10 randomly selected plants of each line making the total collection $50 \times 10 \times 10 = 5000$. The squash technique and pollen fertility test used followed earlier reports (Sheidai *et al.* 2008).

In order to determine a significant difference in cytogenetic characteristics the analysis of variance (ANOVA) followed by the least significant difference test (LSD) as well as χ^2 test were performed on the genotypes. The grouping of the cultivars was performed by using UPGMA (Unweighted Paired Group using Average Method) and NJ (Neighbor-Joining) clustering methods (Podani 2000). For cluster analysis, standardized data (mean=0, variance=1) were used to obtain Euclidean and Taxonomic distances. Statistical analysis used SPSS ver.9 (1998) and NTSYS ver. 2.02 (1998) software.

Results

Diffuse stage in meiosis-I prophase

The meiotic analysis of all cotton cultivars studied showed the occurrence of a post pachytene diffuse stage (Fig. 1A). Despiralization of chromosomes occurred after pachytene, commencing the diffuse stage. The diffuse stage may be of the complete type, in which whole chromosomes decondense, or it may be partial, in which some parts of the genome show decondensation. The present study shows the occurrence of a partial/complete diffuse stage in the cotton cultivars.

Chiasma frequency and chromosome pairing

All cotton cultivars studied showed $2n=4x=52$ chromosome number (Table 1). All cultivars formed mostly bivalents, although some amount of univalents and quadrivalents were also formed (Table 1).

Mehr cultivar showed the highest value of rod bivalents (24.10) followed by the hybrid cultivar Mehr X No. 200 (23.16), while the hybrid cultivar Mehr X Sindose showed the highest value of ring bivalents (3.80) followed by parental cultivar Sahel (3.20).

The highest value of univalents occurred in the hybrid cultivar Mehr X Tabladilla (8.06) while, the lowest value for the same occurred in Mehr cultivar. Similarly the highest value of quadrivalents occurred in the parental genotype Siokra (0.34) followed by hybrid cultivar, Mehr X Sindose (0.29). The other cultivars showed much lower values for the occurrence of quadrivalents (Table 1).

The highest value of total and terminal chiasmata (28.13), occurred in the hybrid cultivar Mehr X Sindose, while the lowest values for the same parameters occurred in Bellizovar (24.75). A very low amount of intercalary chiasmata occurred in some of the genotypes with the highest value in the hybrid cultivar Mehr X Siokra (0.18).

Meiotic abnormalities

Various meiotic abnormalities including, laggard chromosomes (Fig. 1C), stickiness (Fig. 1D),

Fig. 1. Representative meiotic cells in cotton cultivars studied.

A=Diffuse stage (arrows) in the hybrid cultivar Mehr X Tabladila, B=Metaphase I cell showing univalents in the hybrid cultivar Mehr X Sindose. C=Anaphase I laggard in the hybrid cultivar Mehr X Siokra. D=Metaphase I meiosis showing chromosome stickiness in Tabladila. E=Abnormal tetrad in No. 200. Cultivar. F=A tripolar cell in Sindose cultivar. G=Unreduced pollen grain (bigger size pollen grain) in No. 200 cultivar. H and I=Cytomictic cells in the hybrid cultivars Mehr X Belizovar and Mehr X Sindose respectively. J=Aneuploid cell in Siokra. K=Abnormal cell at the end of meiosis in Mehr X No. 200 cultivar. L=Abnormal pollen grain in Mehr X No. 200 cultivar.

Table 1. Meiotic characteristics in cotton cultivars studied

Cultivars	2n	rd	rb	I	IV	IX	TX	TOX
NO:200	52	22.34	2.34	2.23	0.07	0.00	27.34	27.34
SIndose	52	22.34	1.50	3.94	0.10	0.03	25.72	25.75
Mehr	52	24.10	1.23	1.20	0.03	0.00	26.70	26.70
Belizovar	52	21.84	1.27	5.40	0.10	0.00	24.75	24.75
Siokra	52	18.80	3.20	6.63	0.34	0.08	26.45	26.54
Tabladila	52	22.93	1.67	2.45	0.06	0.03	26.61	26.64
Mehr X No. 200	52	23.16	1.55	2.38	0.06	0.00	26.51	26.52
Mehr X Siokra	52	20.10	2.90	5.37	0.15	0.19	26.37	26.56
Mehr X Tabladila	52	19.06	2.77	8.10	0.06	0.06	24.87	24.93
Mehr X Sindose	52	19.35	3.81	4.51	0.29	0.00	28.13	28.13
Mehr X Belizovar	52	22.16	2.00	3.29	0.09	0.01	26.42	26.52

Abbreviations: rd=Rod bivalent, rb=Ring bivalent, I=Univalent, IV=Quadrivalent, IX=Intercalary chiasmata, TX=Terminal chiasmata, TOX=Total chiasmata.

micronuclei formation, unorganized chromosomes and multipolar cell formation (Fig. 1F) were observed in almost all populations studied.

Chromosomes stickiness occurred in both metaphase of meiosis I and II. This occurred be-

tween 2 bivalents or more, forming complete clumps in some cases (Fig. 1D). The highest percentage of metaphase I cells showing chromosome stickiness was observed in Mehr X Siokra (32.40%), while Mehr parental genotype did not show it at all. Mehr X Tabladilla hybrid cultivar was the only genotype showing metaphase II chromosome stickiness (7.00%).

Different degrees of univalent formation to complete desynapsis (all univalents) were observed in the genotypes studied particularly in the hybrid cultivars (Fig. 1B). Chromosomes stickiness during anaphase I and II leading to the formation of anaphase bridges in some of the cases were observed in hybrid genotypes. The number and thickness of these bridges varied among different meiocytes. Laggard chromosomes of different numbers occurred in most of the genotypes with the highest percentage (10) occurring in the Mehr X No. 200 hybrid cultivar. These laggards may be the reason for micronuclei formation in telophase I.

Anaphase abnormalities leading to the formation of multipolar cells (Fig. 1E) were observed in all populations. The highest percentage of triads (Fig. 1F) occurred in the hybrid Mehr X Siokra (0.80%). χ^2 test showed significant difference for meiotic abnormalities among the populations studied.

The occurrence of chromatin/chromosome migration (cytomixis) was observed in meiocytes of the genotypes studied during different stages of meiosis (Fig. 1H, I). The occurrence of large pollen grains (possibly $2n$ pollen grains) was observed along with smaller (normal) pollen grains in all genotypes (Fig. 1G). The large pollen grains comprised about 0.50% of pollen grains in these cultivars. The size of normal (reduced) pollen grains varied from 63.00–101.70 μm , while the size of unreduced ($2n$) pollen grains varied from 87–136 μm in the cultivars studied.

Grouping of the cultivars

Different methods of cluster analysis as well as ordination of the cotton cultivars produced similar grouping (Fig. 2). Some of the parental genotypes and their hybrids formed distinct clusters indicating genetic differences from the other genotypes. For example, Mehr, No. 200 and their hybrid Mehr X No. 200, along with Tabladilla, formed the first major cluster. Similarly Siokra and its hybrid Mehr X Siokra showed affinity and formed a separate cluster to which the hybrid genotype Mehr X Sindose joins with some distance. However, the hybrid cultivar Mehr X Belilzovar joins one of its parental genotypes *i.e.* Belilzovar with some distance. The same holds true for hybrid Mehr X Tabladilla which stands far from both its parents.

Fig. 2. UPGMA tree of cytogenetic data.

Discussion

The occurrence of a diffuse stage has been reported in several plant species (Sybenga 1992). Different reasons suggested for the occurrence of a post pachyten diffuse stage include high synthetic activity analogous to the lampbrush stage in the amphibian oocyte and meiotic arrest to withstand the adverse environmental conditions, *etc.* (Sheidai and Inamdar 1991). At present we do not know the reason for the occurrence of the diffuse stage in cotton, but it occurs in the parental genotypes and their hybrids indicating genetic control over its occurrence.

Significant difference in the chiasma frequency and chromosome pairing among cotton cultivars is considered to be an important genetic feature (Endrizzi *et al.* 1984), indicating genetic difference among cotton genotypes. Such variation in a species/populations with the same chromosome number is considered a means for generating new forms of recombination influencing the variability within natural populations in an adaptive way (Rees and Dale 1974, Rees and Jones 1977).

The cultivars studied showed adjacent quadrivalent formation. The occurrence of different types of quadrivalents has been considered as an important cytogenetic marker differentiating tetraploid cotton cultivars and also revealing the cultivars' genomic differences (Menzel and Brown 1978, Endrizzi *et al.* 1984). Usually cotton cultivars having type I adjacent quadrivalents show high pollen fertility due to proper chromosome segregation as also observed in the present study (>95% pollen fertility was observed in the cultivars studied). Other types of chromosomes orientation, including type II adjacent pairing and alternate orientation, have been reported in some of the cotton cultivars and their hybrids (Menzel and Brown 1978, Endrizzi *et al.* 1984, Sheidai *et al.* 2008). These types of chromosomes pairing occur due to heterozygote translocations among the A genome chromosomes and among D genome chromosomes. In some instances, V-shaped A-D heterozygote translocations with alternate orientation have been reported in Iranian cotton cultivars, for example in the hybrid cultivar Oltan X Sahel and in parental genotypes of modified cultivars (Sheidai *et al.* 2008).

The chromosome stickiness observed in the present study may lead to other cytogenetic abnormalities like laggard chromosome formation, multipolar cell formation and the occurrence of aneuploidy (Baptista-Giacomelli *et al.* 2000). Genetic, environmental factors and their interaction are known to bring about chromosome stickiness in different plant species and cultivars (Baptista-Giacomelli *et al.* 2000).

The multipolar cells observed are most likely due to spindle abnormalities. Such meiotic abnormalities lead to the formation of abnormal tetrads and aneuploid gametes as observed in these cotton genotypes (Villeux 1985, Nirmala and Rao 1996). Migration of chromatin material (cytomixis), observed in some of the cotton cultivars, has also been considered the cause of aneuploid and polyploid gametes formation in plant species and cultivars (Falistocco *et al.* 1995). Cytomixis occurs through cytoplasmic connections originating from the pre-existing systems of plasmodesmata formed within the anther tissues. The plasmodesmata become completely obstructed by the deposition of callose, but in some cases they persist during meiosis and increase in size forming conspicuous inter-meioocytes connections or cytotoxic channels that permit the transfer of chromosomes. The cotton cultivars showing cytomixis also showed abnormal tetrads as well as some degree of pollen infertility and abnormal pollen formation. These abnormalities occur as a result of unbalanced chromosome migration in cytomixis among neighboring meioocytes.

Weber (1934) reported the occurrence of aneuploid male gametes in the species and cultivars of *Gossypium* and related these gametes to the clumping of chromosomes and lagging bivalents. According to Weber, the aneuploid plants in cotton either have been overlooked or they do not exist due to degeneracy or incompatibility of aneuploid gametes. As his observations did not show any degeneracy of aneuploid gametes, he concluded that aneuploid individuals have been over-

looked. The present study shows that aneuploid pollen grains are mostly infertile and therefore may not form aneuploid plants through fertilization.

In some cases cytomixis brings about complete migration of the chromosomes among the neighboring meiocytes forming unreduced gametes (Falistocco *et al.* 1995, Sheidai *et al.* 1999, 2006). The presence of giant pollen grains has been used as an indication of the production of $2n$ pollen (Villeux 1985). Therefore the larger sized pollen grains in the cotton cultivars studied may be considered as potential $2n$ (unreduced) pollen grains. Identification of the cotton genotypes producing unreduced gametes may be of practical use in the cotton hybridization program. This phenomenon seems to be under genetic control as the parental genotypes and their hybrids showed the potential for unreduced pollen grain formation.

Grouping of the parental genotypes and their hybrids in 1 cluster indicates the genomic effects on chromosome behavior, as observed in the Mehr, No. 200 and their hybrid Mehr X No. 200, as well as in Siokra and its hybrid Mehr X Siokra, which showed affinity forming a separate cluster. However, those cases where in the hybrid cultivar stands far from both its parents may indicate different interactions between the 2 parental genomes in the hybrid as was observed in the hybrid Mehr X Tabladila which stands far from its parental genotypes. Therefore different hybrids and crossing progenies may differ in their cytogenetic (partly genomic) characteristics which in turn bring about new genetic variation in the cotton gene pool (germplasm) to be used in cotton breeding.

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